



Evaluation of wound healing activity of *Opuntia Ficus Indica* thron extract on wistar Albino Rat

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Abstract

Traditionally, *Opuntia ficus indica* is used for wound-healing activity. There are currently no detailed scientific data available regarding the wound-healing activity of *Opuntia ficus-indica*. *Opuntia ficus-indica*, the present study was designed to explore the effect of ethanolic thorn extract of *Opuntia ficus indica* (OFI) on experimentally induced excision and incision wound models in Wistar rats. The wound-healing efficacy of ethanolic extracts of *Opuntia ficus indica* was evaluated in excision and incision wound models by topically applying the ethanolic extract in the form of ointment as per the Indian Pharmacopoeia. The extract was formulated in an ointment base at concentrations of 5% and 10% and topically applied to the wounds. In the excision and incision wound model, topical application of different concentrations of *Opuntia ficus indica* extract ointment (5% and 10% w/w extract in the simple ointment) has shown a high rate of wound contraction and decrease in the period of epithelization time and improved the tensile strength to a greater extent when compared to the control (simple ointment treated) group. The 10% extract ointment-treated group demonstrated greater wound-healing-promoting property than the 5% extract ointment-treated animals. In the excision wound model, the animals showed a significant reduction in the period of epithelization and improved wound contraction, whereas the increase in the breaking strength was evident from the incision model. The results suggest that the ethanolic thorn extract of *Opuntia ficus indica* (OFI) possesses wound-healing properties when applied topically.

Keywords: Ethanolic Thron extract, incision, *Opuntia ficus-indica*, wound healing, Tensile strength.

Introduction

Wounds

A wound is defined as a type of injury which that occurs quickly, involving the tearing, cutting, or puncturing of the skin, where blunt force trauma causes a contusion. The integrity of any tissue is may be compromised, such as breaks, muscle tears, burns, or bone fractures). fractures. wound may can result from various causes, including acts such as gunshots, falls, surgical procedure; procedures; disease diseases; or condition. medical conditions. are physical injuries that result disrupt skin, which body's largest organ and primary barrier against external harm. They can range in severity from minor abrasions to deep tissue damage, and their management is essential to prevent complications such as infections, delayed healing, and chronic wounds. Wound healing is a complex and dynamic process that involving physiological mechanisms aimed at restoring the integrity of damaged tissue. [4-5] can are generally into two main types: acute and chronic. Acute wounds, such as cuts, burns, or surgical incisions, typically follow a predictable healing pattern and tend to resolve within a defined period, period when appropriately. In contrast, chronic wounds, such including ulcers, venous leg ulcers, and pressure sores, fail to progress through the normal stages of healing and remain unresolved for longer extended Chronic wounds pose present significant clinical challenge due to their resistance to standard treatment modalities and their impact on the patient's Life of affected individuals. [6-7]

Classification of Wounds: Acute Versus Chronic

Wounds are commonly categorized into two broad groups: acute wounds and chronic wounds, based on their healing trajectory and time frame. Acute wounds are typically caused by trauma or surgery and follow a predictable,

orderly healing process. They progress through the phases of haemostasis, inflammation, proliferation, and remodelling within a relatively short period. Examples of acute wounds include cuts, burns, abrasions, punctures, and surgical incisions. Provided there are no underlying health issues or infections, acute wounds generally heal completely within days to weeks. Chronic wounds are those that fail to heal within the expected timeframe, often persisting for weeks or months. They frequently occur in individuals with underlying conditions such as diabetes, vascular insufficiency, or compromised immune systems. These wounds often remain trapped in the inflammatory phase, preventing progression to subsequent healing stages. Common examples include diabetic ulcers, pressure ulcers (bedsores), and venous leg ulcers. Chronic wounds present a significant clinical challenge due to their resistance to conventional therapies and their detrimental impact on patients' quality of life. [17-18]

Importance of Wound Management

Effective Wound Management

Is essential to ensure proper healing and minimize complications. Poorly managed wounds can result in infections, delayed healing, chronic pain, and more severe systemic issues such as sepsis or amputation. Wound management requires a holistic approach, including thorough wound assessment, cleaning, dressing, infection control, and, when appropriate, the use of advanced therapies such as negative pressure wound therapy (NPWT), growth factors, and regenerative medicine. [19-20]

Phases of Wound Healing

Wound healing is a dynamic and highly regulated process involving a series of overlapping phases that work together to repair damaged tissue. These phases—haemostasis,

inflammation, proliferation, and remodelling—ensure that the wound closes, infection is prevented, and the tissue regains both structural and functional integrity.

▪ Haemostasis

Haemostasis is the initial phase of wound healing, commencing immediately after injury. Its primary goal is to stop bleeding and stabilize the wound environment. Role of Platelets and Clot Formation: When a wound occurs, blood vessels are damaged, exposing collagen and tissue factors. This exposure triggers the aggregation of platelets at the injury site. Platelets adhere to the exposed collagen and release various clotting factors, including fibrinogen, thrombin, and von Willebrand factor, which activate the coagulation cascade.

▪ Inflammatory Phase

The inflammatory phase begins shortly after haemostasis and can last several days. It functions to remove debris, eliminate invading microorganisms, and create an environment conducive to tissue repair.^[22-23]

Materials and Method

Plant Materials: Leaves of *Opuntia ficus indica* were collected from the local area of Dewas.

Preparation of extract

The leaves of *Opuntia ficus-indica* were shade-dried and coarsely powdered. The powder was subjected to Soxhlet extraction using a hydro-alcoholic solvent (70% ethanol and 30% water) for 72 hours. Excess solvent was removed using a rotary flask evaporator, and the crude extract obtained was stored in airtight containers in a refrigerator at temperatures below 10 °C for further studies. The yield (w/w) of the crude thorn extract of *Opuntia ficus indica* was 14.5%.^[19-21]

Preliminary phytochemical tests

Test for Alkaloids

- **Mayer's test:** 5 mg of *Opuntia ficus-indica* extract was transferred into a test tube, and 1% hydrochloric acid (HCl) was added. The resulting solution was gently heated. The appearance of a red colour indicates the presence of alkaloids, as potassium mercuric iodide is present in Mayer's reagent.
- **Wagner's Test:** In this test, 5 mg of *Opuntia ficus-indica* extract was placed in a test tube, and 0.5 mL of Wagner's reagent was added to the solution. The mixture was shaken well. The appearance of a reddish-brown colour indicates the presence of alkaloids. This reddish-brown colour results from the formation of an insoluble complex with iodine.

Test for Flavonoids

Shinoda Test: First, 5 mg of the extract was added to a test tube, followed by a small amount of magnesium. Then, a few drops of concentrated hydrochloric acid were added to the solution. The presence of flavonoids is indicated by a pink colour. Colour variations from orange to red indicate flavones; red to crimson indicate flavonoids; and crimson to magenta indicate flavanones. Catechins, when treated with vanillin solution in hydrochloric acid, produce a red or pink colour.

Test for Glycosides

- **Liebermann's Test – Liebermann's test:** Is used to determine the presence of glycosides in the aqueous

extract of *Opuntia ficus-indica*. In this test, 5 mg of the *Opuntia ficus-indica* extract is thoroughly mixed with 2 ml of chloroform, followed by the addition of 2 ml of acetic acid. The solution is then cooled on ice. After cooling, 1 ml of concentrated sulfuric acid is added. A colour change from violet to green indicates the presence of alkaloids in the extract.

- **Salkowski's test for the analysis of glycosides:** 2 ml of chloroform was mixed with 1 ml of the extract. Then, 2 ml of concentrated sulfuric acid was added and the mixture was shaken gently.

Test for Tannins

Ferric Chloride Test: Five milligrams of an aqueous extract of *Opuntia ficus indica* were mixed with 0.5 ml of ferric chloride solution. The formation of a blackish precipitate indicates the presence of tannins.

Test for saponins: The foam test was performed to identify the presence of saponins in the aqueous extract. In this test, 1 ml of the extract was dissolved in 5 ml of distilled water. After adding the distilled water, the mixture was shaken thoroughly until foam was observed. A few drops of olive oil were then added to the foam, and the mixture was shaken vigorously. The formation of an emulsion indicates the presence of saponins.

Test for Carbohydrates

- **Benedict's test was performed using Benedict's reagent to analyse carbohydrates:** A 5 mg extract was mixed with a few drops of Benedict's reagent and then boiled. The presence of carbohydrates is indicated by a reddish-brown precipitate; however, no such precipitate was observed, indicating the absence of carbohydrates.

Test for steroids: Five milligrams of *Opuntia ficus-indica* extract were mixed with 1 ml of chloroform, followed by the addition of a few drops of concentrated sulfuric acid and acetic acid. The appearance of a greenish colour indicated the presence of steroids.^[27]

Animals: Wistar albino rats, weighing 260–270 g, were obtained from the animal house of the Department of Pharmacology at Swami Vivekanand College of Pharmacy, Indore, India. The animals were housed four per cage, allowed free access to water and food, and maintained at a constant temperature (23 ± 1 °C) and humidity (60 ± 10%) under a 12-hour light/dark cycle (lights on from 07:30 to 19:30). Animal treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985).^[25]

A total of 20 rats were divided into the following groups

- Group I: Control (Normal Saline, 2 mL/kg)
- Group II: Standard Treatment (Povidone-Iodine Ointment)
- Group III: Test (5% aqueous extract of *Opuntia ficus-indica* thorns)
- Group IV: Test (10% aqueous extract of *Opuntia ficus-indica* thorns)

Preparation of Ointment

Test samples of OFI were prepared using an ointment base. The simple ointment was formulated by melting weighed quantities of white wool fat (2.9 g), yellow soft paraffin

(40.25 g), Ceto stearyl alcohol (2.9 g), and liquid paraffin (2.9 g). Leaf extract of *OFI* was then incorporated into the ointment base at concentrations of 5% and 10% by mixing thoroughly on an ointment slab using a spatula. The formulated ointments were stored in a refrigerator for subsequent wound healing studies.^[26]

Experimental Design

A total of 20 rats were divided into the following groups:

Group I: Control (Normal Saline, 2 mL/kg)

Group II: Standard Treatment (Povidone-Iodine Ointment)

Group III: Test (5% aqueous extract of *Opuntia ficus-indica* thorns)

Group IV: Test (10% Aqueous extract of *Opuntia ficus-indica* thorn)

Procedure: Wistar albino rats, weighing between 260 and 270 grams and of either sex, were selected for the experiment.

1. Prior to the experiment, the rats were randomly divided into four groups. Each group contained five rats.
2. The first group was treated as the control group (normal saline).
3. The second group was treated with standard povidone-iodine ointment.
4. The third and fourth groups were treated with aqueous extracts of *Opuntia ficus-indica* thorns at concentrations of 5% and 10%, respectively.^[23]

Wound Healing Activity

Excision and incision wound models were used to evaluate wound healing activities.

Excisional Wound Model: On day zero, the animals were anesthetized with 2% Lignocaine Hydrochloride gel and placed on the operating table in their natural position. An impression was made on the central dorsal thoracic region, 5

mm away from the ears, using a round seal with a diameter of 2.5 cm, as described by Morton and Malone (10). The skin within the marked area was excised to full thickness to create a wound approximately 500 mm² in size, using pointed forceps and iris scissors. Haemostasis was achieved by packing the wound with gelatin foam soaked in normal saline solution. The animals were then returned to their individual cages. They were treated with ointments containing different concentrations of aqueous extract until complete epithelialization occurred. On the 11th day post-wounding, epithelial tissue was collected from the fully epithelialized wounds of the rats, and their tensile strength was measured. The remaining epithelialized tissues were dried in a desiccator under vacuum. The dried tissues were weighed, and hydroxyproline and total protein content were estimated.^[23-24]

Result and Discussion

Acute Toxicity: The LD 50 of the extract was found to be 2000 mg/kg.

Table 1. Indicating presence of various photochemical constituents.

S.No.	Test	Positive/ Negative
1	Carbohydrate	+
2	Terpenoids	-
3	Flavone Glycoside	+
4	Phenolic Compound	+
5	Flavonoids	+
6	Saponins	-
7	Sterols	+

Note: -+ = Present

- = Absent

1. Wound Healing Activity
2. Excisional wound model

Table 2: Effect of aqueous extract of *Opuntia ficus indica* thorn for Excisional wound model. Wound area measurement (mm²) and percentage wound contraction in excisional wound healing model.

Treatment	0 Day	4 th Day	8 th Day	12 th Day	16 th Day	20 th Day
Control	512±3.1	478.20±6.9 (9.23)	320.15±4.9 (51.71)	244.18±5.2 (69.25)	145.25±3.6 (87.28)	45.34±2.9 (97.78)
Povidone-iodine	514±4.8	322.71±6.7** (37.43)	99.8±6.1** (82.19)	0.813±0.11** (99.82)	-	-
Extract 1 (5%)	524±3.9	399.41±5.4 (22.64)	209.03±4.2 (59.51)	60.42±3.3 (88.29)	7.81±1.7 (98.48)	-
Extract 2 (10%)	518±3.4	389.21±6.21 (25.24)	162.33±1.9 (68.35)	42.06±2.8 (91.68)	2.99±0.13 (99.42)	-

All value is given in mean±SEM, **P* < 0.05, ***P* < 0.01 as compare with the control group (one-way ANOVA followed by Dunnett's test).^[18]

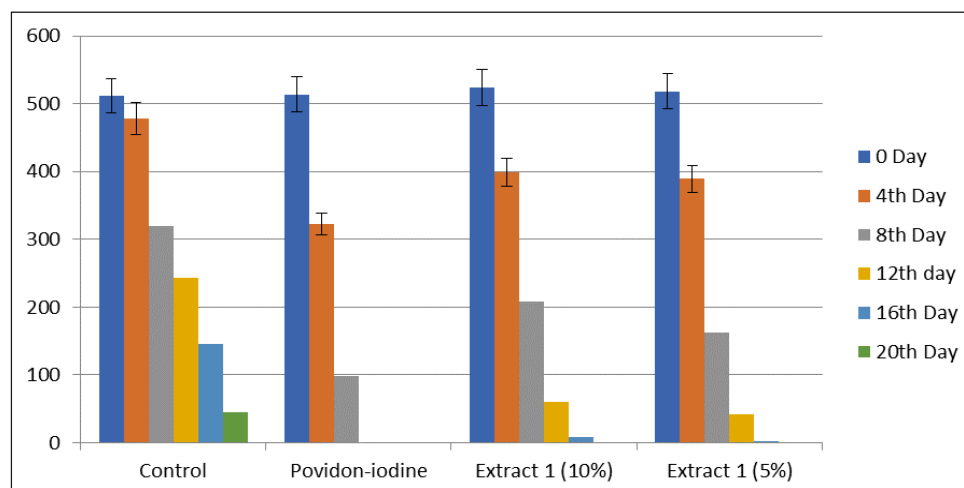


Fig 1: Effect of aqueous extract of *Opuntia ficus indica* thorn for Excisional wound model. Wound area measurement (mm²) and percentage wound contraction in excisional wound healing model.

Summary and Conclusion

The current study demonstrated that a 10% ointment formulated from the aqueous extract of *Opuntia ficus indica* exhibits significant wound-healing properties. This is evidenced by a marked increase in the rate of wound contraction, a reduction in the epithelization period, and enhanced tissue tensile strength. Additionally, hydroxyproline and protein content were significantly elevated. Hydroxyproline measurement serves as an indicator of collagen turnover, and the increased breaking strength of granulation tissue suggests improved collagen maturation, likely due to enhanced cross-linking.

Thus, it is suggested that the wound healing activity of the aqueous extract of *Opuntia ficus indica* Thron is related to the presence of flavonoids and vitamin C. In conclusion, the study's results indicate that the aqueous extract of *Opuntia ficus indica* Thron. accelerates wound healing by enhancing epithelialization and collagen deposition, providing scientific evidence for the traditional use of *Opuntia ficus-indica*.

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